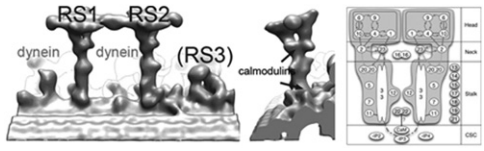
orientation and rotational information of the cargo at the pausing moments. Recently, we introduced the single particle orientation and rotational tracking (SPORT) technique to follow the rotational motion of plasmonic gold nanorods (References: 1. J. Am. Chem. Soc., 2010, 132, 16417. 2. ACS Nano, 2010, 4, 7667. 3. J. Am. Chem. Soc., 2011, 133, 5720.). In the present work, we acquired for the first time the orientation and rotational information of the cargo during both the moving (directional transport) and pausing stages of axonal transport with a high temporal resolution of 2 ms. The lateral and rotational motions of cargos during directional transport and pausing stages are elucidated, and their relationship with motor protein competition and regulation is discussed. The simultaneous tracking of lateral movement and rotational motions of nano-objects also shadows a new insight into the dynamic function of living cells.

193-Plat
Crowding of Molecular Motors Determines Microtubule Depolymerization
Louis Reese, Anna Melbinger, Erwin Frey.
Arnold Sommerfeld Center for Theoretical Physics and Center for NanoScience, Department of Physics, Ludwig-Maximilians-Universität München, Munich, Germany.
Assembly and disassembly dynamics of microtubules (MTs) is tightly controlled by MT associated proteins. Here, we investigate how plus-end-directed depolymerases of the kinesin-8 family regulate MT depolymerization dynamics. Employing an individual-based model, we reproduce experimental findings. Moreover, crowding is identified as the key regulatory mechanism of depolymerization dynamics. Our analysis gives two qualitatively distinct regimes. For motor densities above a particular threshold, a macroscopic traffic jam emerges at the plus-end and the MT dynamics become independent of the motor concentration. Below this threshold, microscopic traffic jams at the tip arise which cancel out the effect of the depolymerization kinetics such that the depolymerization speed is solely determined by the motor density. Because this density changes over the MT length, length-dependent regulation is possible. Remarkably, motor cooperativity does not affect the depolymerization speed but only the end-residence time of depolymerases.

194-Plat
Networks of Dynein and Regulatory Proteins in Flagella/Cilia Visualized by Electron Cryo-Tomography
Takashi Ishikawa, Khanh Huy Bui, Gaia Pigino.
Paul Scherrer Institute, Villigen PSI, Switzerland.
Eukaryotic flagella/cilia are bending apparatus with >300 proteins. Sliding between microtubule doublets by dynein motors, which forms inner (waveform determination) and outer (accelerator) arms, is motility force. However, integration of linear dynein motility into well-organized bending is unclear. To address this question, localization and interaction of dynein and regulatory proteins are essential. We reconstructed structure of flagella/cilia at pseudo-atomic resolution utilizing electron cryo-tomography. We located the positions of eight major inner dyneins from 11 known in Chlamydomonas genome as well as other dyneins which exist locally in flagella (minor dyneins): at the proximal 2 micron area or on one microtubule doublet. This could explain asymmetric waveform with a sharp kink. We also reconstructed the radial spoke (RS), which are supposed to regulate by calcium (Pigino et al. (2011) JCB, in press). Chlamydomonas has two RSs, while Tetrahymena has three. Interestingly Chlamydomonas also has a short protrusion at the position of Tetrahymena RS3, which resembles RS3, suggesting evolutional pathway. Mutant structures revealed connection of 23 component proteins (figure). Calmodulins are localized at the branch of bifurcation and the bottom, close to the interface between RS and dynein tail.



Platform: Molecular Dynamics I

195-Plat
Probing the Energy Landscape of Ribosome Function Through Simulation
Paul C. Whitford, Karissa Sanbonmatsu.
Los Alamos National Laboratory, Los Alamos, NM, USA.
The ribosome is a massive ribonucleic protein complex (>2 MDa), composed ofover 50 polymer chains, and is the sole producer of proteins in the cell. It undergoes many large-scale conformational rearrangements (including ~ 100 Å displacements) that allow for transfer RNA molecules to read messenger RNA. These complex rearrangements enable rapid translation (1 amino acid per 5-100 milliseconds) and high fidelity (error rates of ~1:1000). The vast information available about static ribosome structures and the ribosome's overall dynamics make it the model system for establishing the physical relationships between the energetics and biological function. We use explicit-solvent simulations of the complete ribosome (2+ million atoms) to bridge the gap between experimentally-measured kinetics and the free-enery landscape. For tRNA entry into the ribosome (i.e. accommodation), we have obtained 2.1 microseconds of sampling, with multiple runs exceeding 200ns each. To provide the first insights into the diffusive dynamics associated with large-scale 'ratcheting' rearrangements, which are essential for tRNA movement through the ribosome (translocation), we have performed a continuous 1 microsecond simulation, which required ~4 months on 2,048 cores. The ribosome appears to exhibit diffusive properties in particular reaction-coordinate spaces, which is consistent with these coordinates being accurate descriptors of translocation-associated rearrangements. Together, this work is allowing for the first comparison of experimental kinetics to estimates of barriers that can be obtained theoretically, or experimentally.

196-Plat
Single-Molecule Studies of DNA Self-Diffusion in Entangled Blends of Linear and Circular DNA
Cole D. Chapman[1], Sachin Shanbhag[2], Douglas E. Smith[1], Rae M. Robertson-Anderson[3].
[1]UCSD, San Diego, CA, USA, [2]FSU, Tallahassee, FL, USA, [3]USD, San Diego, CA, USA.
Using fluorescence microscopy and single-particle tracking we have measured the self-diffusion coefficients for single DNA molecules in varying blends of entangled linear and relaxed circular (ring) DNA. We have determined the dependence of self-diffusion of both species on the fraction of linear vs. ring species in the blend, the overall solution concentration, and the molecular length. The diffusion of relaxed circular DNA (D_R) was found to depend strongly on the fraction of linear DNA (ϕ_L) in the solution with D_R undergoing a dramatic decline as ϕ_L was incrementally increased to ~ 0.3, followed by a much slower decline as ϕ_L approached 1. This phenomenon can be attributed in part to the tendency of linear polymers to thread their circular counterparts, prohibiting diffusion via reptation and forcing ring DNA to diffuse via the slow mechanism of constraint release. For linear DNA, a surprising non-monotonic dependence of self-diffusion (D_L) on ϕ_L was observed with D_L reaching a minimum at ϕ_L ~ 0.6. This behavior was not anticipated by previous theoretical models, but the results are in good agreement with predictions from new simulations using a minimal constraint release model that assumes our exact experimental conditions. Molecular entanglements are essential for observing these dramatic effects on diffusion within DNA blends, as we have found that below the critical threshold for molecular entanglement there exists only a weak correlation between self-diffusion and blend composition.

197-Plat
Molecular Dynamics and Calcium Binding Studies on Troponin C
Steffen Lindert, Peter Kekenes-Huskey, J. Andrew McCammon.
UCSD, La Jolla, CA, USA.
Troponin C (TnC) is part of the myofilament complex in both cardiac and skeletal muscle cells. It initiates myofilament contraction upon calcium binding. Here we focus on elucidating the dynamics of the cardiac regulatory domain of TnC in response to calcium as well as troponin I (TnI) binding. Both classical and accelerated molecular dynamics simulations are performed on an apo, a calcium-bound and a calcium-TnI-bound system. Conformational sampling between the two simulation methods is compared. Using a principle component analysis the conformations sampled by the different system are compared. NMR order parameters and chemical shifts are calculated based on the simulations. Agreement with experimental observables suggests that a substantial amount of the protein's native dynamics is sampled by the simulations. Brownian dynamics simulations of calcium association with TnC on representative structures from the molecular dynamics simulations diffusion limited calcium association rates in agreement with experimental measurements. The gating regime for calcium association is investigated using Brownian dynamics simulations on representative intermediate structures.